

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Lawrence P. Wackett, et al.

Serial No.: Not yet assigned (Division of 09/155,036)

Filed: Even date herewith

For: DNA MOLECULES AND PROTEIN DISPLAYING IMPROVED TRIAZINE
COMPOUND DEGRADING ABILITY

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

The present application is a division of U.S. Patent Application No. 09/155,036, filed on 17 September 1998, which in turn is a 371 filing of International Patent Application No. PCT/US98/00944, filed 16 January 1998, which in turn claims the benefit of U.S. Provisional Patent Application No. 60/035,404, filed 17 January 1997.

Prior to taking up the above-identified application for examination, please amend the application as follows:

IN THE SPECIFICATION

Please insert the following two new paragraphs at page 1, line 4, immediately before the heading, "Background of the Invention":

-- STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support from the United States Department of Agriculture-BARD program, Grant No. 94-34339-112. The government may have certain rights in this invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a division of U.S. Patent Application No. 09/155,036, filed on 17 September 1998, which in turn is a 371 filing of International Patent Application No. PCT/US98/00944, filed 16 January 1998, which in turn claims the benefit of U.S. Provisional Patent Application No. 60/035,404, filed 17 January 1997, all of which are hereby incorporated

herein by reference in their entirety. --

Please replace the paragraph beginning at page 7, line 21, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

-- **Fig. 1.** Nucleotide sequence alignment of wild type *atzA* (bottom sequence) from *Pseudomonas sp.* strain ADP and clone (A7) (SEQ ID NO:1 and SEQ ID NO:3). The boxed sequences indicate areas of nonidentity between the two nucleotide sequences. --

Please replace the paragraph beginning at page 7, line 24, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

-- **Fig. 2.** Nucleotide sequence alignment of wild type *atzA* (bottom sequence) from *Pseudomonas sp.* strain ADP and clone (T7) (SEQ ID NO: 1 and SEQ ID NO:4). The boxed sequences indicate areas of nonidentity between the two nucleotide sequences. --

Please replace the paragraph beginning at page 7, line 27, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

-- **Fig. 3.** Amino acid sequence alignment of wild type AtzA (bottom sequence) from *Pseudomonas sp.* strain ADP and clone (A7) (SEQ ID NO:2 and SEQ ID NO:5). The boxed sequences indicate areas of nonidentity between the two amino acid sequences. Start, indicates beginning of the protein; Stop, indicates end of the protein. --

Please replace the paragraph beginning at page 7, line 30, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

-- **Fig. 4.** Amino acid sequence alignment of wild type AtzA from *Pseudomonas sp.* strain ADP and clone (T7) (SEQ ID NO:2 and SEQ ID NO:6). The boxed sequences indicate areas of nonidentity between the two amino acid sequences. Start, indicates beginning of the protein; Stop, indicates end of the protein. --

Please replace the paragraph beginning at page 8, line 1, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

-- **Fig. 5.** Nucleotide sequence alignment of wild type *atzA* (SEQ ID NO:1, bottom sequence) from *Pseudomonas sp.* strain ADP and clone (A11). Fig. 5(a) provides the sequence from nucleic acids 11-543 (SEQ ID NO:7), Fig. 5(b) provides the sequence from nucleic acids 454-901 (SEQ ID NO:8), Fig. 5(c) provides the sequence from 1458-1851 (SEQ ID NO:9; N in this sequence indicates that this nucleotide has not been verified) and Fig. 5(d) provides the sequence from nucleic acids 1125-1482 (SEQ ID NO:10) of clone A11. The boxed sequences indicate areas of nonidentity between the two nucleotide sequences. The "N" in these sequences refer to nucleic acids that are being verified. The four "C" nucleotides depicted above the top sequence in 5(a) and the eleven "G" nucleotides depicted above the top sequence in 5(b) indicate the correct nucleotide sequence of the top sequence. --

Please replace the paragraph beginning at page 26, line 22, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

-- Recursive sequence recombination was performed by modifications of existing procedures (Stemmer, W., Proc. Natl. Acad. Sci. USA 91:10747-10751 (1994) and Stemmer, W. Nature 370:389-391 (1994)). The entire 8.4 kb plasmid was treated with DNAase I in 50 mM Tris-Cl pH 7.5, 10 mM MnCl₂ and fragments between 500 and 2000 bp were gel purified. The fragments were assembled in a PCR reaction using Tth-XL enzyme and buffer from Perkin Elmer, 2.5 mM MgOAc, 400 μM dNTPs and serial dilutions of DNA fragments. The assembly reaction was performed in an MJ Research "DNA Engine" thermocycler programmed with the following cycles:

- | | |
|---|--|
| 1 | 94°C, 20 seconds |
| 2 | 94°C, 15 seconds |
| 3 | 40°C, 30 seconds |
| 4 | 72°C, 30 seconds + 2 seconds per cycle |
| 5 | go to step 2 39 more times |
| 6 | 4°C -- |

IN THE CLAIMS

Please cancel claims 1 and 12-22 as requested in the Request for Filing A Divisional Patent Application Under Rule §1.53(b), filed concurrently herewith.

09/155,036

REMARKS

Claims 1 and 12-22 are canceled without prejudice or disclaimer. Claims 2-11 and 23-34 are now pending.

The specification is amended at page 1 to add a statement regarding government rights and to add a cross reference to the prior application.

With regard to the amendments to pages 7 and 8 of the specification, it was noted that features depicted in the Figures as filed in the priority document, PCT/US98/00944, filed January 16, 1998, were not described in the specification as filed. The Brief Description of the Drawings for Figures 1 through 5 has been amended to describe these features.

The specification is amended at page 26 to correct a typographical error.

Applicant respectfully submits that these amendments embody merely the correction of formal matters in the specification, and respectfully requests that the preliminary amendment described herein be entered into the record prior to examination and consideration of the above-identified application.

Respectfully submitted,
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I hereby certify that this paper and/or fee is/are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box Patent Application, Washington, D. C. 20231.

Marc Ireland
Name: Marc Ireland

APPENDIX A
SPECIFICATION / CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE

Applicant(s): Lawrence P. Wackett, et al.
Serial No.: Not yet assigned
Filed Even Date Herewith
DNA MOLECULES AND PROTEIN DISPLAYING IMPROVED
TRIAZINE COMPOUND DEGRADING ABILITY
Attorney Docket No. 110.00440102

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. In addition, all amendments have been bolded.

In the Specification

The paragraph beginning at page 7, line 21, has been amended as follows:

Fig. 1. Nucleotide sequence alignment of wild type *atzA* (bottom sequence) from *Pseudomonas sp.* strain ADP and clone (A7) (SEQ ID NO:1 and SEQ ID NO:3). **The boxed sequences indicate areas of nonidentity between the two nucleotide sequences.**

The paragraph beginning at page 7, line 24, has been amended as follows:

Fig. 2. Nucleotide sequence alignment of wild type *atzA* (bottom sequence) from *Pseudomonas sp.* strain ADP and clone (T7) (SEQ ID NO: 1 and SEQ ID NO:4). **The boxed sequences indicate areas of nonidentity between the two nucleotide sequences.**

The paragraph beginning at page 7, line 27, has been amended as follows:

Fig. 3. Amino acid sequence alignment of wild type AtzA (bottom sequence) from *Pseudomonas sp.* strain ADP and clone (A7) (SEQ ID NO:2 and SEQ ID NO:5). **The boxed sequences indicate areas of nonidentity between the two amino acid sequences. Start, indicates beginning of the protein; Stop, indicates end of the protein.**

The paragraph beginning at page 7, line 30, has been amended as follows:

Fig. 4. Amino acid sequence alignment of wild type AtzA from *Pseudomonas sp.* strain ADP and clone (T7) (SEQ ID NO:2 and SEQ ID NO:6). **The boxed sequences indicate areas**

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The paragraph beginning at page 26, line 22, has been amended as follows:

Recursive sequence recombination was performed by modifications of existing procedures (Stemmer, W., *Proc. Natl. Acad. Sci. USA* 91:10747-10751 (1994) and Stemmer, W. *Nature* 370:389-391 (1994)). **[[Mervyn, do you know more now about what was done?]]** The entire 8.4 kb plasmid was treated with DNAase I in 50 mM Tris-Cl pH 7.5, 10 mM MnCl₂ and fragments between 500, and 2000 bp were gel purified. The fragments were assembled in a PCR reaction using Tth-XL enzyme and buffer from Perkin Elmer, 2.5 mM MgOAc, 400 μM dNTPs and serial dilutions of DNA fragments. The assembly reaction was performed in an MJ Research "DNA Engine" thermocycler programmed with the following cycles:

- 1 94°C, 20 seconds
- 2 94°C, 15 seconds
- 3 40°C, 30 seconds
- 4 72°C, 30 seconds + 2 seconds per cycle
- 5 go to step 2 39 more times
- 6 4°C